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Addendum

Arabidopsis Co-Repressor Complexes Containing Polyamine Oxidase-Like Proteins and Plant-Specific Histone Methyltransferases

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Addendum to:

AtCZS, an* Arabidopsis *C2H2 Zinc Finger-SET Histone Methyltransferase, is a Plant-Specific Chromatin Modifier

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ABSTRACT

Regulation of genes by repression of transcription represents a virtually universal mechanism that underlies such diverse biological processes as restriction of expression of neuronal genes to neurons in mammals, and control of flowering in plants. However, while the molecular mechanisms of transcriptional gene silencing in animal systems are being intensively studied, our understanding of these processes in plants is very sparse and, because plants often utilize unique strategies to establish and maintain chromatin states, only limited use can be made of information available on epigenetic modifications in nonplant systems.

Our recent work identified two *Arabidopsis* factors, a SWIRM domain polyamine oxidase (PAO)-like protein, AtSWP1, and a plant-specific C2H2 zinc finger-SET domain histone methyltransferase (HMT), AtCZS, that interact with each other in plant cells and repress target gene(s) by histone hypoacetylation and generation of heterochromatic histone methylation marks. Thus, AtSWP1 and AtCZS were suggested to represent two main components of a corepressor complex. Here, we discuss these finding in the context of our knowledge of animal corepressor complexes, speculate on potential components of the AtSWP1/AtCZS corepressor complex that are unique to plants, and propose a working structure/function model of *Arabidopsis* PAO/HMT-containing corepressor complexes.

In eukaryotic cells, core histones undergo a variety of covalent modifications, which determine the structure and activity of the corresponding chromosomal region.[1] These modifications promote conversion of the local chromatin into euchromatic or heterochromatic state, thus enhancing or restricting transcriptional activity, respectively, and are generated by chromatin-modifying complexes, which incorporate numerous enzymatic activities required for the specific modifications.[2,3] Comprised of a large number of proteins and executing either transcriptional activation or repression tasks,[2] these complexes play a crucial role during animal and plant growth and development.[4,5] Yet, while this aspect of the eukaryotic cellular biology has been extensively investigated in animals, the studies of plant chromatin-modifying complexes are just beginning. Joining these research efforts, we have identified a novel type of transcriptional repressor complex which may act as one of the global regulators of plant gene expression and which combines both general, i.e., conserved between plants and animals, and plant-specific corepressor activities and components.[6]

MAMMALIAN POLYAMINE OXIDASE (PAO)/LYSINE DEMETHYLASE 1 (LSD1)-CONTAINING COREPRESSOR COMPLEX

One of the well-studied examples of transcriptional repressors in animals is a multiprotein corepressor complex that specifically silences neuronal genes in nonneuronal cells (Fig. 1, inset). During mammalian development, a variety of neuronal genes, such as ion channels and neurotransmitter receptors, are silenced in nonneuronal tissues to allow correct cellular differentiation. A large group of these repressed genes, of which probably the best-known example is the type II voltage-dependant sodium channel,[7,8] contain within their DNA sequence a 23 bp-long regulatory element, designated repressor element-1 (RE1).[9,10] RE1 is directly recognized and bound by RE1-silencing transcription factor (REST),[11] the DNA binding domain of which is represented by N-terminal C2H2-type zinc fingers motifs.[8,11] Notably, REST does not possess any enzymatic activity and serves as a gene-targeting factor for the corepressor complex, while other components of the complex provide the actual enzymatic modules that covalently modify histones and repress expression of the target neuronal genes. These enzymatic activities

include (1) histone deacetylases (HDACs) 1 and 2 which are thought to exist in complex[12] with a REST-interacting protein CoREST;[13] (2) LSD1, a protein containing two hallmark domains, SWIRM [Swi3p, Rsc8p, Moira[14]] that may anchor the tail of the histone H3 molecule,[15] and PAO (polyamine oxidase)-like that acts as a histone H3 lysine demethylase;[16] and (3) G9a, a SET [*Su(var)3-9, Enhancer-of-zeste, Trithorax*[17]]-domain protein with histone methyltransferase (HMT) activity.[18,19] Besides these major components, other proteins, e.g., REST-associated mSin3, methyl-DNA binding factor $MeCP_2$, etc.,[20,21] copurify with the PAO/LSD1-containing corepressor complexes (Fig. 1, inset).

Because the REST protein recognizes a unique RE1 sequence within the target gene(s), it makes biological sense that, instead of REST, other DNA binding zinc finger proteins may associate with the mammalian LSD1-containing corepressor complexes to direct them to gene groups with different response elements. Indeed, a zinc finger protein ZNF217 was shown to associate with CoREST/LSD1 complexes[12,22] and bind a distinct 15 bp-long consensus recognition sequence (CRS) within the human E-cadherin promoter[22] (Fig. 1, inset).

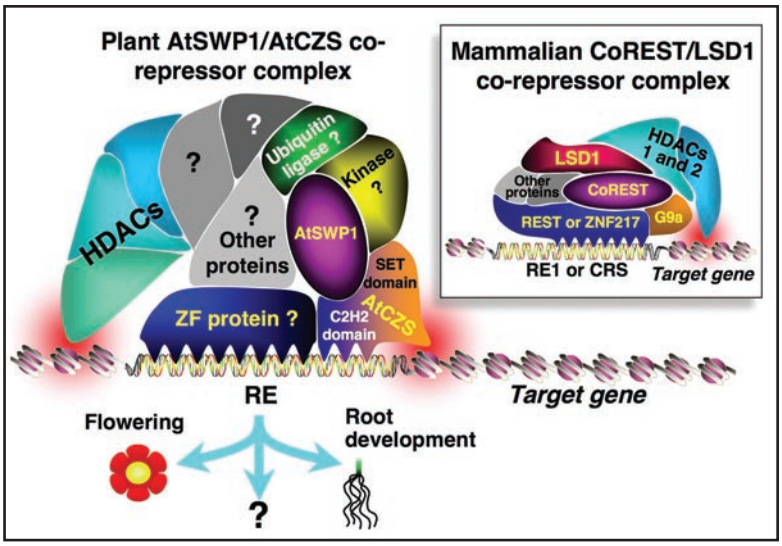

Figure 1. Main components of the mammalian versus plant PAO-containing corepressor complexes. See the text for details.

PLANT PAO-CONTAINING CO-REPRESSOR COMPLEX AND PLANT-SPECIFIC HISTONE METHYLTRANSFERASE

Despite the importance of histone modifications in determination of the chromatin state and, therefore, cell fate during plant development,[23,24] our knowledge about plant corepressor complexes in general, and PAO-containing corepressor complexes in particular, is virtually nonexistent. Only relatively recently an existence of a gene repression mechanism involving PAO-like protein has been provided by an observation that a plant homolog of the *LSD1* gene, termed *FLOWERING LOCUS D* (*FLD*), represses, via histone hypoacetylation, *FLOWERING LOCUS C* (*FLC*), a negative regulator of flowering;[25] the histone modifying machinery involved in the FLD-mediated repression of *FLC*, however, remains unknown. Unlike the composition of the protein complexes that modify plant histones, the histone modifications that index active or inactive chromatin are relatively well characterized. Specifically, *Arabidopsis* chromatin contains H3K4/H3K36 and H3K9/H3K27 methylation marks indexing active and inactive chromatin, respectively.[26-31] Also, histone acetylation in plants has been characterized,[32,33] and the family of plant HDAC proteins includes, based on sequence analysis of the *Arabidopsis* genome, three major groups: PD3/HDA1 and SIR2 conserved in eukaryotes and a plant-specific HD2 family.[34]

Recently, we have isolated two main components of a novel chromatin-modifying corepressor complex from *Arabidopsis thaliana*: —a SWIRM-domain PAO-like (SWP) protein AtSWP1, and a plant-specific C2H2 zinc finger PreSET-SET-PostSET-domain (CZS) protein AtCZS that likely acts as HMT (Fig. 1)—and demonstrated that they directly interact with each other in-planta (Fig. 2) and are required for generation of the H3K9 and H3K27 methylation marks on heterochromatin.[6] AtCZS contains SET domain diagnostic of HMTs;[35] thus, it presumably represents the actual enzyme that methylates H3K9 and H3K27 whereas the effect of AtSWP1 on these histone methylation marks is indirect, most likely through recruiting AtCZS. Because genetic inactivation of AtSWP1 and/or AtCZS alters not only histone methylation, but also acetylation,[6] the

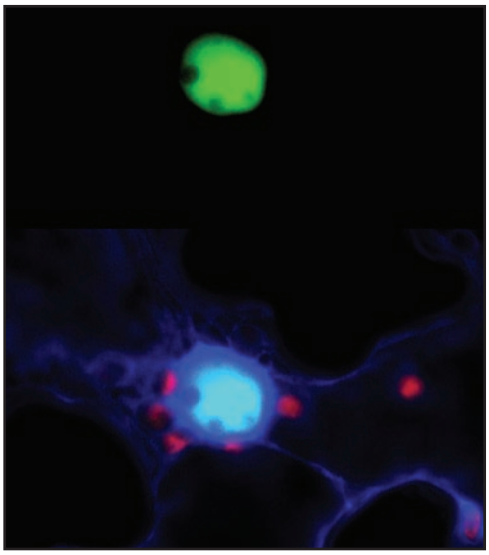

Figure 2. In planta interaction between AtSWP1 and AtCZS as detected by bimolecular fluorescence complementation (BiFC). (A) Reconstituted fluorescence of N-terminal and C-terminal halves of YFP fused to the interacting AtSWP1 and AtCZS proteins, respectively. (B) Merged images of the reconstituted YFP signal, co-expressed free CFP that identifies the cell nucleus and outlines the expressing cell, and plastid auto-fluorescence YFP signal is in green, CFP signal is in dark blue, overlapping YFP and CFP signals are in light blue, and plastid autofluorescence is in red. Images are a single confocal section. For further detail, see reference 6.

AtSWP1/AtCZS corepressor complex most likely also contains as yet unidentified HDACs (Fig. 1).

Interestingly, AtCZS represents a unique aspect of plant corepressor complexes because of the combination of the SET and C2H2 zinc finger domains within the same protein molecule. Although conserved between evolutionarily-distant plant species such as monocots and dicots,[6] it is found only in plants.[35] In mammalian PAO-containing complexes,[36] the AtCZS functionality is most closely represented by one of their zinc finger proteins, REST[11,37] or ZNF217,[12] and the PreSET-SET-PostSET HMT G9a protein that

methylates H3K9 and/or H3K27.[18,19] Thus, AtCZS likely combines the functionalities of the two different animal corepressor complex components in a single molecule (Fig. 1). In this scenario, AtCZS, similarly to REST or ZNF217, may play a role in initial association of the corepressor complex with regulatory elements of its target genes in the plant genome. Alternatively, the C2H2 zinc finger domain of AtCZS may simply augment and stabilize the interaction between the AtSWP1/AtCZS corepressor complex and its target gene whereas the binding specificity may be provided by another, as yet unidentified, DNA binding protein.

Another potential difference between the plant and animal PAO-containing corepressor complexes may lie in the functionality of the PAO-like protein itself. While both animal LSD1 and plant AtSWP1 exhibit a common conserved domain structure, i.e., N-proximal SWIRM domain and C-proximal PAO-like domain,[6,16] only LSD1 possesses a detectable demethylase activity when tested in vitro using the purified recombinant proteins.[6,16]

A WORKING MODEL OF THE ATSWP1/ATCZS COREPRESSOR COMPLEX

Our working model of the AtSWP1/AtCZS corepressor is based on the notion that it may combine both general and plant-specific activities and components. For example, the presence of a PAO-like protein, SET-domain HMT, zinc finger proteins, and HDACs would represent a general feature, plant and animal. On the other hand, the specific PAO-like protein AtSWP1 may lack the demethylase activity, and the AtCZS is a plant-specific HMT which is not found in animal complexes. It is tempting to speculate that another potential plant-specific feature of the AtSWP1/AtCZS complex may be association with plant-specific HD2 HDACs. Moreover, plant corepressors may contain multiple species of the AtSWP protein family, which comprises four members in *Arabidopsis*,[16] and one of these proteins may be a functional histone demethylase. Finally, plant complexes may contain novel functions, such as kinases and ubiquitin ligases (Fig. 1), which have been identified to bind AtSWP1 in our ongoing yeast two-hybrid protein interaction screens (unpublished data). Because histones are known to undergo phosphorylation[38] and ubiquitination,[39] it is possible that the AtSWP1/AtCZS corepressor complex may be able to affect an exceptionally wide spectrum of histone modification, from methylation/demethylation to deacetylation, to ubiquitination and to phosphorylation, making it a truly multifunctional chromatin modifier.

Another important and still unresolved aspect of the AtSWP1/AtCZS complex is the range of its target genes. To date, we have documented one AtSWP1/AtCZS target gene, the *FLC* negative regulator of flowering, and the corresponding flower timing delay phenotypes of the *Arabidopsis* null mutants in *AtSWP1* and *AtCZS*.[6] However, these mutants exhibited other, unrelated to *FLC*, phenotypic changes, such as altered root length, indicating existence of additional target genes of the AtSWP1/AtCZS complex (Fig. 1). Indeed, our microarray analyses of both mutant lines identified over 70 genes the expression of which was upregulated 4–8 fold in the mutants as compared to the parental, wild-type plants (unpublished data). A significant subset of these upregulated genes, including those predicted to participate in root development, was shared between the two mutants whereas no genes were detected that showed significant down-regulation in either of the mutants. These observations are consistent with the role of AtSWP1 and AtCZS as gene repressors acting in concert in the same multiprotein complex. Furthermore, they suggest a role for the AtSWP1/AtCZS corepressor complex in global regulation of plant gene expression.

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